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Serum opsonic capacity against *Yersinia enterocolitica* O:3 in yersiniosis patients with or without reactive arthritis

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SUMMARY

The opsonic capacity of 45 sera from patients with reactive arthritis after *Yersinia enterocolitica* O:3 infection and of 45 matched sera from yersiniosis patients without post-infection complications was studied at 1–3 months, 5–8 months and 12–20 months after the onset of the infection. Antibody-mediated opsonization of virulence-plasmid-containing *Y. enterocolitica* O:3 was studied by measuring complement-fixation on opsonized bacteria and opsonophagocytic function of the polymorpho-nuclear leucocytes (PMN). The PMN response against bacteria pre-opsonized by heat-inactivated sera was measured by using a chemiluminescence (CL) assay. The fixation of complement C1q and C3 on bacteria was determined by flow cytometry using fluorescein-conjugated C1q- and C3c-antisera. All the sera were strongly opsonic at the onset of the infection, and this capacity persisted in most of the patients still at the end of the follow-up. No difference was observed in complement-fixing capacity between the sera of the two groups, but the sera from arthritic patients showed stronger augmentation of PMN CL response at the early phase of the infection ($P=0{\cdot}005$ in the presence of complement, $P=0{\cdot}04$ in the absence of complement). These results suggest that enhanced opsonic capacity may play a role in the development of *Yersinia*-triggered reactive arthritis by leading to strong activation of the PMN and, consequently, to release of inflammatory mediators.

Keywords *Yersinia enterocolitica* reactive arthritis opsonization IgA complement

INTRODUCTION

The acute disease typically caused by *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* is gastroenteritis. It may be followed by post-infection complications such as reactive arthritis. These complications are strongly associated with the HLA-B27 tissue antigen (Aho *et al.*, 1974). Interesting characteristics of immune response to *Yersinia* have been observed in patients with reactive arthritis when compared to those with no post-infection manifestations. Arthritis patients have stronger and persisting antibody response especially of IgA class against the pathogen (Toivanen *et al.*, 1987b), and avidity of IgM class *Yersinia* antibodies is weaker while that of IgA class is stronger (Lahesmaa-Rantala *et al.*, 1987b). They show weaker cell-mediated immune response against the microbe (Vuento *et al.*, 1983) and prolonged occurrence of circulating *Yersinia*-containing immune complexes in their sera (Lahesmaa-Rantala *et al.*, 1987a). Persistence of the etiological microbe somewhere in the tissues of patients with *Yersinia*-triggered reactive arthritis has been suggested (Toivanen *et al.*, 1985; Toivanen *et al.*, 1987a, Toivanen & Toivanen, 1988).

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Virulence of *Y. enterocolitica* and *Y. pseudotuberculosis* is dependent on a 65–70 kb plasmid (Portnoy *et al.*, 1984). This plasmid codes for outer membrane protein structures (YOPs and V-antigen) (Bölin, Portnoy & Wolf-Watz, 1985) and is associated with attachment to epithelial cells (Vesikari *et al.*, 1981), resistance to killing activity of serum (Pai & DeStephano, 1982), resistance to phagocytosis (Lian & Pai, 1985) and inhibition of complement-mediated opsonization (Tertti *et al.*, 1987). Loss of the virulence-plasmid leads to avirulence of these bacteria (Gemski, Lazere & Casey, 1980). Immune response during the *Yersinia* infection is directed against both plasmid-coded and chromosome-coded structures (Granfors, Viljanen & Toivanen, 1981; Simonet *et al.*, 1985; Ståhlberg *et al.*, 1987). Anti-*Yersinia* antibodies found in patient sera overcome the inhibition of complement-mediated opsonization, which is a virulence factor of *Yersinia*, but antibodies against the virulence-plasmid-coded structures are not an absolute prerequisite for this (Tertti *et al.*, 1988).

Since our previous findings indicate the importance of antibodies in defence against *Yersinia*, in the present work we have studied the opsonic capacity of sera from patients with yersiniosis without any complications and from yersiniosis patients developing reactive arthritis. We demonstrate that at an early phase of disease patients with reactive arthritis have

stronger opsonic capacity than yersiniosis patients who do not develop any post-infection complications. We suggest that this reflects stronger activation of host defence mechanisms at the onset of *Yersinia* infection, which might contribute to the development of the post-infection arthritis.

MATERIALS AND METHODS

Bacterial culture conditions

A plasmid-containing clinical isolate of *Y. enterocolitica* O:3 was used as antigen. The strain was positive in an autoagglutination test indicating presence of the virulence-plasmid (Laird & Cavanaugh, 1980) and expression of the YOP1 (Skurnik *et al.*, 1984). The bacteria were grown in calcium-deficient medium and expression of YOPs in the bacterial preparation was verified by immunoblotting as described previously (Tertti *et al.*, 1987).

Patients

The sera studied are from a patient material reported previously by our group (Lahesmaa-Rantala *et al.*, 1987b). They have been collected from patients with clinically typical *Yersinia* infection confirmed by bacteriological or serological evidence. To compare the serum opsonic capacity of *Y. enterocolitica* O:3 patients with non-complicated disease and of patients with reactive arthritis as a post-infection complication, 90 sera matched for disease duration were tested. Twenty pairs were examined at 19–100 days from the onset of the gastrointestinal disease, 10 pairs at 5–8 months after onset of the disease, and 15 pairs at 12–20 months. Thirty-six (80%) out of the 45 sera in the reactive arthritis group and two (4%) out of 45 sera in the non-arthritic group were from B27-positive patients. In addition, 11 patient sera containing predominantly one immunoglobulin class of anti-*Yersinia* antibodies were included.

Measurement of Yersinia *antibodies*

IgM, IgG and IgA class antibodies against *Yersinia* were measured by EIA as described previously (Granfors *et al.*, 1980). SDS-extract of whole *Y. enterocolitica* O:3 bacteria was used as antigen.

Cell preparation

PMN were isolated from healthy individuals as described previously by using dextran sedimentation and Ficoll-Paque gradient centrifugation (Tertti *et al.*, 1987). The final concentration of PMN was $2{\cdot}0 \times 10^6$/ml in Hanks' balanced salt solution without phenol red but supplemented with 0·1% gelatin (gel-HBS).

Opsonization

Serum used as a fresh complement source was collected from a healthy B27-negative and *Yersinia* antibody negative individual and stored immediately at −70°C. The patient sera were inactivated prior to use in the opsonization assay at 56°C for 30 min. Stored bacteria were thawed and washed with phosphate-buffered saline supplemented with 0·1% gelatin (gel-PBS) at 4°C. Bacteria were pre-opsonized for 30 min at 4°C in 10% patient serum and washed with gel-PBS. The pre-opsonized bacteria were then used in the chemiluminescence assay in the presence or absence of complement. For complement fixation studies opsonization of the bacteria was carried out in gel-HBS containing 2·5% opsonizing serum (vol/vol) at 37°C on a shaker, 250 rev/min. After opsonization the bacteria were washed and resuspended to gel-PBS.

Chemiluminescence (CL) assay

A luminol-dependent CL assay was modified for measurement of opsonic capacity of antibodies against *Y. enterocolitica* O:3 as described previously (Tertti *et al.*, 1988). The method is highly specific for *Yersinia* antibodies. The assay was started by adding to 0·5 ml of PMN suspension 0·1 ml of suspension of pre-opsonized bacteria and complement to a final concentration of 2·5% (vol/vol). The CL was measured by a luminometer (Luminometer 1251, Wallac, Turku, Finland) for 60 min. The results are presented as peak values of the PMN CL responses.

Complement fixation studies

The flow cytometry method described previously (Tertti *et al.*, 1987) and modified for the estimation of C1q and C3 on opsonized bacteria (Tertti *et al.*, 1988) was used. This method detects specifically the opsonic capacity of *Yersinia* antibodies as shown by our previous works. C1q- and C3-fixation were determined by using fluorescent antibodies (fluorescein-conjugated anti-human-C1q and fluorescein-conjugated anti-human-C3c, Behringwerke AG, Marburg, FRG). Fluorescence of individual bacteria was analysed by using an EPICS-C flow cytometer (Coulter Electronics, Hialeah, FL, USA). The results for the fluorescence of single stained bacteria are presented as fluorescence histograms.

*Purification of IgG and IgA class anti-*Yersinia *antibodies*

Fractions containing only IgG or IgA class antibodies to *Yersinia* were prepared from sera of two patients. They had typical clinical features of *Yersinia* infection, *Yersinia enterocolitica* O:3 was isolated from the stool of the other one (1455/85), and they had high levels of IgM, IgG and IgA class antibodies in the serum. The sera used for this study were from the acute phase of infection taken at 2 weeks (277/86) and 6 weeks (1455/85) after the onset of infection.

IgG and IgA antibody fractions were prepared by gel filtration and with affinity column chromatography. The serum was applied to Bio-Gel A 5 m column (200/400 mesh, Bio Rad Laboratories, Richmond, CA). Chromatography was performed at 4°C in PBS, pH 7·4. Antibodies to *Yersinia enterocolitica* O:3 in each fraction were determined by class-specific EIA (Granfors, 1980). The column fractions were combined into three pools: an IgM pool (which was not used), IgG pool and IgA pool. IgG and IgA pools were further purified by affinity chromatography. Commercial anti-IgM, anti-IgG and anti-IgA antisera (Dako Immunoglobulins, Copenhagen, Denmark) were conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden), and the matrices were placed in columns. The IgG pool was applied to anti-IgM and anti-IgA columns and the IgA pool to anti-IgM and anti-IgG columns, and fall-through fractions were collected in PBS. The fractions were concentrated by ultrafiltration with Novacell™ filter (Filtron Corporation, Clinton, MA). Concentrations of IgG and IgA class *Yersinia* antibodies in the preparations were finally tested with EIA.

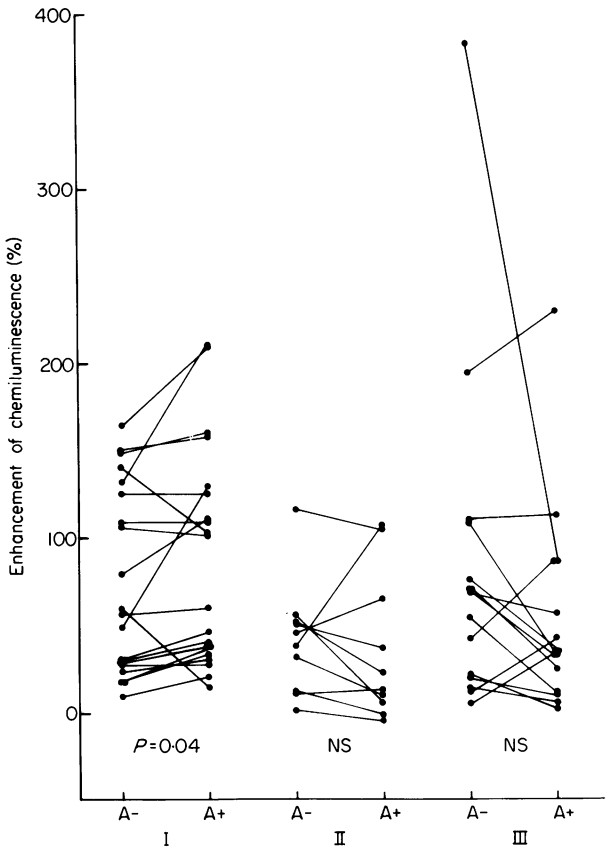

Fig. 1. Effect of antibodies on the PMN CL response in the absence of complement in *Y. enterocolitica* O:3 patients without reactive arthritis, (A−) and *Y. enterocolitica* O:3 patients with reactive arthritis (A+) at 19–100 days (I), 5–8 months (II), and 12–20 months (III) from the onset of the infection. Each dot represents one patient. Each pair of matched sera connected with the line were tested simultaneously by using the PMN from the same donor. For statistical analysis Student's paired *t*-test was used. NS not significant.

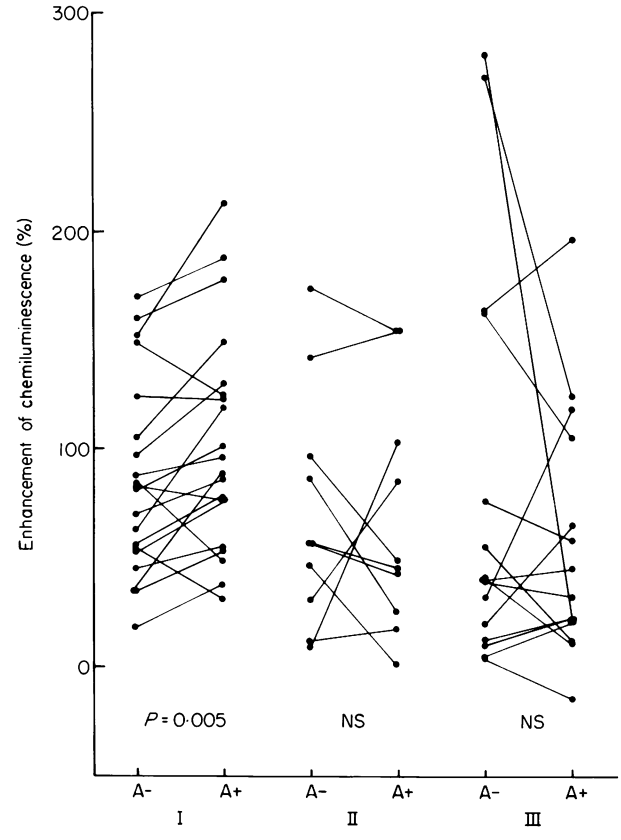

Fig. 2. Effect of antibodies on the PMN CL response in the presence of 2·5% *Yersinia*-antibody-negative complement source. Abbreviations are as in the legend to Fig. 1.

RESULTS

Capacity of the patient sera to enhance the PMN opsonophagocytic response

One to three months after the onset of *Yersinia* infection the sera obtained from reactive arthritis patients were more opsonic than sera from non-complicated cases both in the presence of 2·5% complement ($P=0·005$) and in the absence of fresh complement ($P=0·04$) (Figs. 1 and 2). In the presence of complement only four out of 20 and in the absence of complement three out of 20 of the matched pairs showed superior opsonization by the non-arthritic serum than by the arthritic one. The difference between the arthritic and non-arthritic patients was not observed in the samples taken at 5–8 months and at 12–20 months of infection. B27-positivity did not seem to be a decisive factor, because exclusion of the B27-negative arthritic patients or the B27-positive non-arthritic patients did not change the results. The apparent overlap between the groups is attributable to differences between the PMN used as indicator cells, which are isolated from different healthy donors. Thus, numerical values are not comparable from experiment to experiment due to differences in responsiveness of PMN, but in repeated experiments the relative opsonic capacities of the matched sera were constant. The matched samples must be tested simultaneously, and in statistical analysis a paired test is necessary to avoid the problems of day to day variation.

Table 1. Effect of antibodies on complement fixation on bacteria

	C1q fixation (Buffer = 69 ± 13)			C3 fixation (Buffer = 91 ± 22)		
	I	II	III	I	II	III
A+	330 ± 125	138 ± 73	108 ± 39	525 ± 115	333 ± 149	267 ± 142
A−	301 ± 114	156 ± 95	131 ± 61	507 ± 120	322 ± 173	324 ± 201

The results are presented as mean fluorescences ± s.d. of fluorescence histograms from EPICS-C flow cytometer. A− *Y. enterocolitica* O:3 patients without reactive arthritis, A+ *Y. enterocolitica* O:3 patients with reactive arthritis. I 19–100 days; II 5–8 months; III 12–20 months from the onset of the infection.

One to three months after the infection, the phagocytosis promoting capacity of sera from both arthritic and non-arthritic patients was remarkably strong ($P < 10^{-7}$ when compared to the given value 100% of gel-PBS buffer), and decreased thereafter. However, even at 12–20 months after the infection it was clearly detectable ($P < 0·002$) regardless of the post-infection complications.

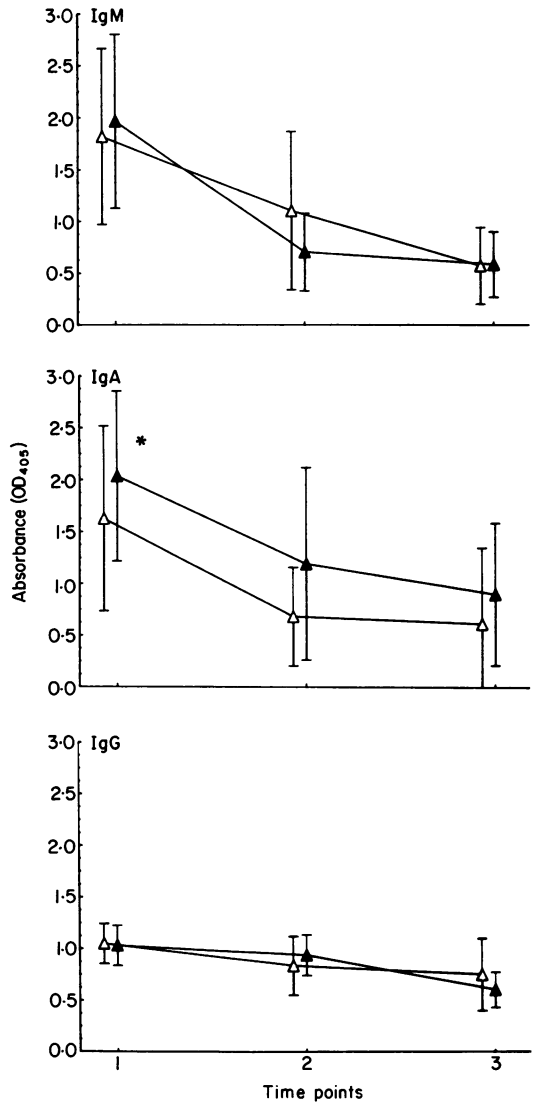

Fig. 3. EIA results of antibodies against *Y. enterocolitica* O:3 in the patient sera 19–100 days (1), 5–8 months (2), and 12–20 months (3) from the onset of the infection. *Y. enterocolitica* O:3 patients without reactive arthritis (△), *Y. enterocolitica* O:3 patients with reactive arthritis (▲), $*P=0{\cdot}02$, Student's paired *t*-test. Mean values ± s.d. are given.

Capacity of the patient sera to fix complement on opsonized bacteria

Unlike in opsonophagocytosis, complement-fixing capacities of sera from arthritic and nonarthritic patients did not differ at any stage of disease. The sera taken 1–3 months after the onset of the infection fixed C1q and C3 effectively (Table 1). Decrease in opsonic capacity was seen during the progress of disease, but complement-fixing capacity persisted at a definite level still at 12–20 months.

EIA results of the patient sera

The patients with reactive arthritis showed stronger anti-*Yersinia* IgA antibody response than those without this complication ($P=0{\cdot}003$), and the difference was most notable at the onset of the infection ($P=0{\cdot}02$) (Fig. 3).

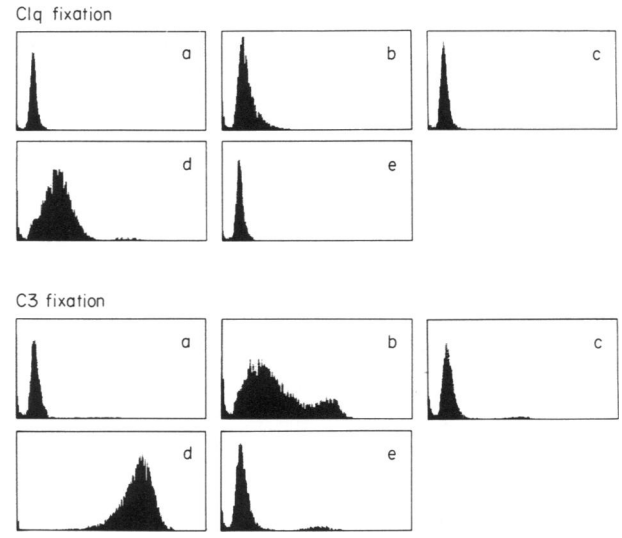

Fig. 4. Flow cytometry results of the effect of purified immunoglobulin preparations on the C1q- and C3-fixation. Gel-PBS control (a), 1455/85 IgG purified (b), 1455/85 IgA purified (c), 277/86 IgG purified (d), 277/86 IgA purified (e). In each frame, the abscissa represents the fluorescence intensity and the ordinate represents the number of individual bacteria.

Opsonic function of Yersinia *antibodies of different isotypes*

The results are summarized in Table 2. From experiments with purified anti-*Yersinia* immunoglobulins and non-treated sera it is evident that antibodies of IgG class are effective in the opsonization of *Y. enterocolitica* O:3 (Table 2, Fig. 4). Most, but not all, patient sera containing predominantly anti-*Yersinia* IgM in the EIA showed strong complement-fixing and phagocytosis promoting capacity. The purified IgA preparations were practically non-opsonic, but the two patient sera with anti-*Yersinia* IgA dominating were clearly positive. However, these two patient sera also had a notable amount of anti-*Yersinia* antibodies of other isotypes.

DISCUSSION

This study demonstrates that in the sera taken early in the course of *Y. enterocolitica* O:3 infection opsonic capacity is stronger in patients with reactive arthritis than in patients with non-complicated disease. We suggest that this may contribute to the pathogenesis of reactive arthritis. Another interesting finding is persistence of opsonic capacity still at the end of the follow-up regardless of the complications. As opsonic and protective capacity are correlated in many cases (Griffiss *et al.*, 1987) and antibodies have been protective in a mouse model of *Yersinia*-infection (Une & Brubaker, 1984), yersiniosis patients might be protected against a reinfection over long periods. In fact, our clinical experience of several hundred patients over the past 10 years is that repeated *Yersinia* infections in the same patient are rare despite the relatively high frequency of the infection in Finland.

This study once again demonstrated that reactive arthritis patients have stronger antibody response of IgA class against *Yersinia* than yersiniosis patients without this complication. It has been suggested that IgA would be dysopsonic due to its poor complement-fixing capacity (Edebo, Richardson & Feinstein,

Table 2. Effect of serum IgG, IgM and IgA class *Yersinia* antibodies on opsonization of *Y. enterocolitica*

	EIA*			Complement fixation†		Chemiluminescence‡	
Patient sera	IgM	IgA	IgG	C1q	C3	C′ absent	C′ 2·5%
With IgM response dominating							
116/84	0·459	0·229	0·194	88	158	100	110
965/85	0·477	0·279	0·305	186	208	182	146
899/86	0·738	0·203	0·160	82	138	92	101
92/85	0·926	0·184	0·214	95	303	110	132
71/85	1·399	0·204	0·231	76	258	90	115
With IgA response dominating							
586/84	0·187	0·464	0·383	114	167	110	124
1496/84	0·412	1·200	0·412	262	365	123	149
1455/85 purified	0·157	1·328	0·166	86	109	88	120
277/86 purified	0·184	1·618	0·102	82	123	103	93
With IgG response dominating							
866/85	0·326	0·320	0·508	94	180	138	137
1193/86	0·329	0·152	0·800	121	332	178	150
777/85	0·407	0·243	0·887	126	338	134	167
1374/84	0·382	0·381	1·029	154	409	159	194
1455/85 purified	0·240	0·178	1·760	110	240	165	114
277/86 purified	0·220	0·107	2·402	195	564	206	137
gel-PBS ($n=14$)	ND	ND	ND	81±20	117±23	100	100

* With *Yersinia enterocolitica* O:3 as the antigen, absorbance values are given.

† Complement fixation results are presented as mean fluorescences of fluorescence histograms from EPICS-C flow cytometer (mean of two experiments).

‡ Chemiluminescence values are presented as percentages of daily gel-PBS-control (mean of two experiments).

C′ complement.

ND not done.

1985; Magnusson *et al.*, 1979; Griffiss, 1975). This led us to test opsonic capacity of sera from *Y. enterocolitica* O:3 patients.

Our results do not support the hypothesis that weaker opsonic capacity would be the cause of ineffective elimination of the microorganism in *Yersinia*-triggered reactive arthritis. Rather, our findings are contrary. We did not, however, observe enhanced complement-fixing capacity in the arthritic patients despite higher phagocytosis-promoting activity. This suggests that a remarkable portion of antibodies in the arthritic sera actually do not activate complement effectively. This reasoning is also supported by our data with purified antibody preparations, as IgA preparations were unable to fix complement.

How could strongly opsonic antibody response contribute to the development of reactive arthritis? It is possible that in arthritic patients the interplay between the PMN and opsonized bacteria is overly active due to unusually strong opsonization of the microorganism. The activated PMN could then consequently contribute to the development of the post-infection joint inflammation by releasing inflammatory mediators. In fact, overactivity of PMN chemotaxis has been seen in association with the B27 tissue type (Leirisalo *et al.*, 1980). This would not explain the involvement of the joint, however, unless the putative hiding microbial products are found in the joint as in *Chlamydia*-triggered arthritis (Keat *et al.*, 1987). Another explanation is that enhanced opsonic activity in sera of reactive arthritis patients secondarily reflects some other process, e.g. at the gut level, which would be the key to the development of complications in *Yersinia* infection.

In conclusion, we detected enhanced opsonic capacity in association of *Yersinia*-triggered reactive arthritis, which may lead to stronger PMN activation and, consequently, to release of inflammatory mediators.

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